Attorney's Docket No.: 86-292002 / MGH-0952.1 Mill

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Miller, S. I.
Serial No. : 09/068,804
Filed : May 14, 1998
Title : SALMONELLA SECRETED PROTEINS AND USES THEREOF

Art Unit : 1641
Examiner : Padma Basker

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.131

I, Samuel Miller, declare as follows:

1. I am the sole inventor of the subject matter claimed in the patent application identified above.
2. I have reviewed an Office Action mailed October 14, 1999, in which the examiner rejected (i) claims 1-4, 7, 10 and 16-18 as anticipated by Kaniga et al. (Journal of Bacteriology, 177, 3965-3971, 1995), and (ii) claims 1-2, 4, 6, 8, 10 and 16-18 as anticipated over Hermant et al. (Molecular Microbiology, 17, 781-789, 1995).
3. I am informed and believe that the earliest date the Kaniga et al. reference was available to the public was July 8, 1995.
4. I am informed and believe that the earliest date the Hermant et al. reference was available to the public was September 18, 1995.
5. The invention claimed in this patent application was conceived and reduced to practice in the United States prior to the publication date of Kaniga et al. and Hermant et al.

CERTIFICATE OF MAILING BY FIRST CLASS MAIL

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

April 14, 2000
Date of Deposit

Tom M Sause
Signature

Tom M Sause
Typed or Printed Name of Person Signing Certificate

6. A copy of the Hueck et al. reference (*Molecular Microbiology* 18, 579-490, 1995) is attached (Attachment A). This reference describes my work related to the claimed subject matter of the present application (see, in particular Figure 4, on page 483 of the article, which discloses the amino acid sequences of SspA/C/D/A). The Hueck et al. manuscript was received by the publishers of *Molecular Biology* on May 17, 1995, and the revised and final version of the manuscript was in the hands of the publisher on or before July 6, 1995. The date the manuscript was revised is shown in the Hueck paper at page 479, column 1.

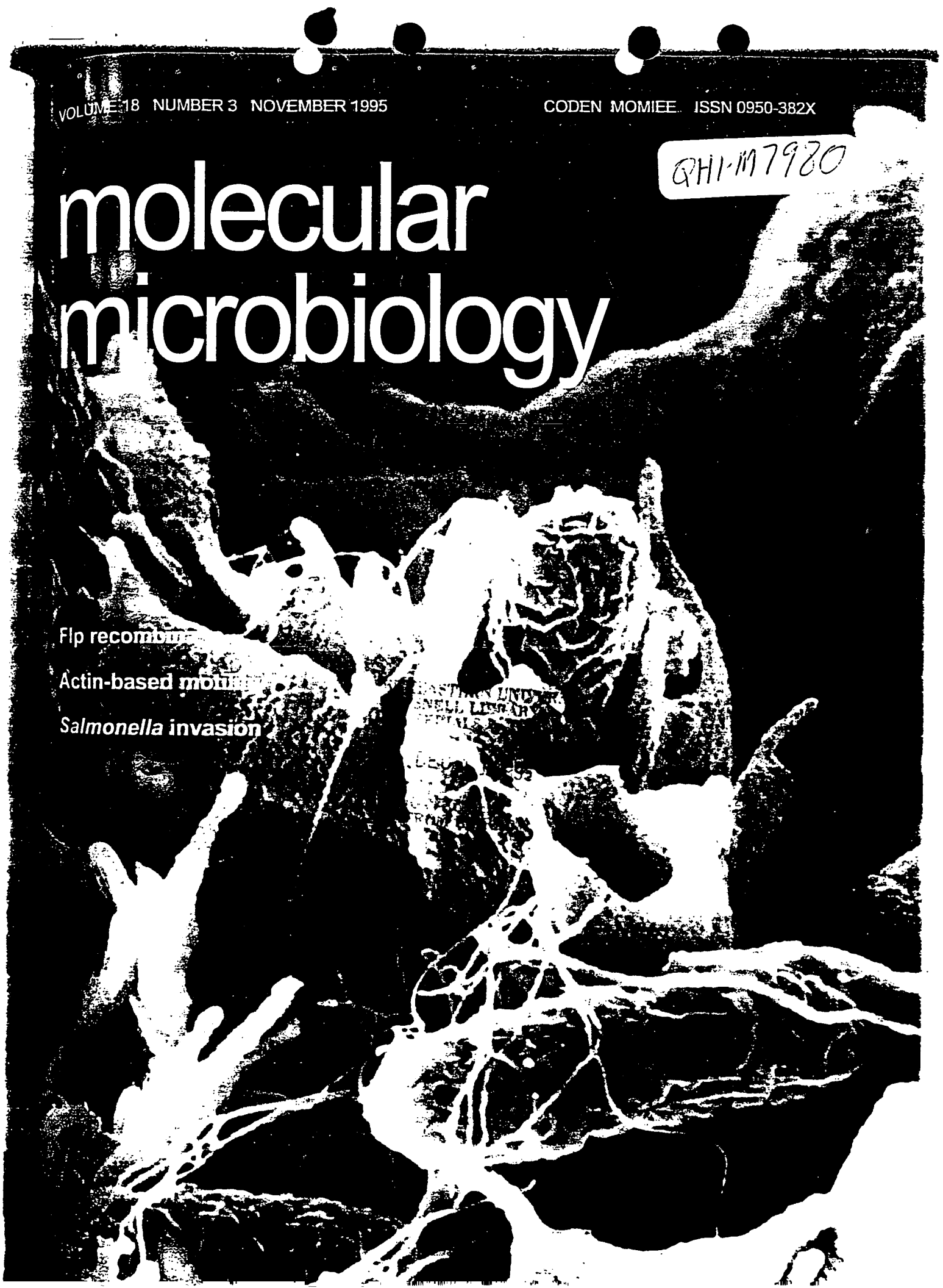
7. I hereby declare that all statements made herein of my own knowledge are true and that all statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued therefrom.

Samuel I. Miller, M.D.

April 11, 2000
Date

VOLUME 18 NUMBER 3 NOVEMBER 1995 CODEN MOMIEE ISSN 0950-382X

molecular microbiology



Flp recomb

Actin-based mobil

Salmonella invasion

Molecular Microbiology (1995) 18(2), 185–189

MicroReview

Molecular handles on adaptive mutation

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Summary

In one experimental system, several handles on the molecular mechanism of apparent adaptive mutation have emerged. The system is reversion of a *lac* frameshift mutation in *Escherichia coli*. The molecular handles include a requirement for homologous recombination; the implication of DNA double-strand breaks as a molecular intermediate; a unique sequence spectrum of −1 deletions in mononucleotide repeats which implies polymerase errors, and also implies a failure of post-synthesis mismatch repair on those errors; and the involvement of sexual functions at some stage of the process. These molecular handles are revealing an unexpected new mechanism of mutagenesis.

Introduction

[illegible] and Delbrück (1943) described mutations in growing populations of bacteria. These mutations, and those described by Lederberg and Lederberg (1952), arose before cells were exposed to a selection for the mutations. In contrast, adaptive mutations (Cairns *et al.*, 1988; Cairns and Foster, 1991; Foster, 1994; reviewed by Foster, 1993) or stress-ful-lifestyle-associated mutations (SLAM; Rosenberg, 1994) are detectable in non-growing cell populations, only on exposure to a non-lethal genetic selection, and have so far been found so far only in genes whose functions were required (but see Hall, 1990). The latter may be Lamarckian. It would be important to know if this were the case.

In one experimental system, an understanding of the molecular mechanism of the adaptive mutagenesis is crystallizing. From the vantage point of a completely unravelled molecular mechanism, one will be able to address the important evolutionary implications of the existence of adaptive mutations. It will be easier to tell what adaptive mutations are when we know how they work. The system about which the most is known, is reversion of a *lac* frameshift mutation carried on an F′ plasmid in *Escherichia coli*. This system will be the exclusive subject of this review. (For an exhaustive review of many systems refer to Foster (1993), and see Maenhaut-Michel and Shapiro (1994) for new molecular information on mutations occurring under selection in a different system whose mechanism of mutation is different, at least in part, from that described here.) Recent advances in understanding the molecular mechanism of adaptive reversion in the *lac* frameshift system are discussed in detail elsewhere (Rosenberg, 1994; Rosenberg *et al.*, 1995). These will be summarized here, the new pieces of information added, and a possible fit for all the pieces of information will be considered.

The assay system

The assay system is a +1 frameshift mutation in *lacI*, which is fused to *lacZ* such that the frameshift is polar on *lacZ* and confers a Lac$^-$ phenotype. This frameshift mutation is carried on an F′ episome in cells deleted for the chromosomal *lac* operon. When plated on minimal lactose medium, growth-dependent, Luria–Delbrück mutants appear initially, on the second day after plating. Over the following week of plate incubation, adaptive revertants also appear, increasing in number linearly each day (Cairns and Foster, 1991).

Recombination

Formation of the late Lac$^+$ revertants requires genes encoding the RecA (Cairns and Foster, 1991) and RecBC proteins of the RecBCD pathway of homologous recombination, whereas formation of the early revertants does not (Harris *et al.*, 1994). In recombination, RecBC enzyme prepares single-strand DNA which is then coated by RecA protein in preparation for invasion of a homologous duplex DNA molecule (see Rosenberg and Hastings, 1991). Thus, both proteins work to catalyse formation of heteroduplex recombination intermediates such as Holliday junctions.

The RecA and RecBC proteins also function in the induction of the SOS system of DNA damage repair. It is argued elsewhere that recombination, and not SOS induction, is the relevant function of these proteins in adaptive mutation (Rosenberg, 1994), and this argument is further

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AMERICAN
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Journals

2 March 2000

Ms. Fran Sheehan,
Library Services
Fish & Richardson, P.C.
222 Franklin Street
Boston, MA 02110

Dear Ms. Sheehan:

The official publication date (i.e., mailing date) of the July-2 (#14) 1995 issue of the *Journal of Bacteriology* was 8 July 1995.

Sincerely,

Linda M. Illig
Director, Journals

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